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Thyroid Control over Biomembranes. VII. Heart Muscle Mitochondria from L-Triiodothyronine-Injected Rats*

Frederic L. Hoch

The University of Michigan Medical School,
Departments of Internal Medicine and Biological Chemistry,
7696 *Kresge Building I, Ann Arbor, Michigan* 48109, *U.S.A.*

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F. L. Hoch. Thyroid Control over Biomembranes. VII. Heart Muscle Mitochondria from L-Triiodothyronine-Injected Rats. *Journal of Molecular and Cellular Cardiology* (1982) **14**, 81–90. Mitochondria prepared from the myocardia of rats injected with L-triiodothyronine, 1 μg/g body weight daily for 3 days, were compared with those from euthyroid or hypothyroid animals. The heart weights of the hormone-treated animals increased 37%, whereas mitochondrial yield remained unchanged. Oxidative phosphorylation measured at 25°C featured a decreased rate of phosphorylating (State 3) respiration with normal phosphorylative efficiency (ADP:O ratio). The respiratory deficit was connected with a progressive loss as the mitochondria stood in iced suspensions; control mitochondrial respiration decreased 0.26-times as fast. The lipid composition of the heart mitochondria of the thyrotoxic group was abnormal in that the excess of neutral lipids (probably triglycerides) had a high content of oleoyl residues. Their phospholipid group compositions and the fatty acyl contents of each of the phospholipid classes were similar to control compositions, in contrast to the excess linoleoyl and decreased arachidonoyl contents of the phosphatidylcholines in the hypothyroid group. Thus the abnormalities of heart mitochondrial function are connected with different patterns of lipid changes in hyperthyroidism and hypothyroidism. Evidence for a relationship of organelle lipids to the reported abnormal myocardial contractile performance in these thyroid states is discussed.

Key Words: Myocardial mitochondria; Phosphorylating respiration; Hyperthyroidism; Membrane lipids; Thermal instability.

Introduction

In the heart mitochondria of hypothyroid rats, the activation energy (E_a) of energy-linked respiration (State 3 or State 4, but not uncoupled respiration) between 37 and 21°C was three times greater than the E_a in mitochondria from control rats [*19*, *36*]. This abnormality of membrane-dependency of oxidative phosphorylation was accompanied by an altered membrane fatty acid composition, as has been found in several experimental conditions [*34*]. Mitochondrial phospholipid oleoyl (18:1) fatty acyl content was depleted and linoleoyl (18:2) content was increased. One injection of L-triiodothyronine or L-thyroxine corrected the thermotropic respiratory responses and the 18:1 acyl content over 3 days, but in addition depleted arachidonoyl (20:4) acyl content and depressed respiratory control measured at 32°C.

In view of the known changes in myocardial contractile performance, especially in response to temperature [*7*], we have examined the lipid composition and oxidative phosphorylation in the heart mitochondria of thyrotoxic rats. Thyroid pretreatments of euthyroid rats are reported to affect heart mitochondrial function differently according to the schedule of administration. For example, six daily injections of 0.1 μg of triiodothyronine/g body weight into male rats decreased State 3 respiration and phosphorylative efficiency at 25°C [*32*], whereas 20 days of feeding triiodothyronine [*32*] or 15 daily injections of 0.35 μg/g into female rats [*28*] increased the rate of fully coupled State 3 respiration by inducing proliferation of respiratory assemblies. We injected male rats with 1.0 μg of triiodothyronine/g/day

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for 3 days, a schedule that should have increased the BMR by about 35% [17] and induced hepatic mitochondrial cytochrome synthesis [23]. To determine if thyroid hormone excess and deficiency produced opposite deviations from normal patterns of lipid composition, as they did with the activities of heart mitochondrial isocitrate dehydrogenase, succinate dehydrogenase and glycerolphosphate dehydrogenase, and the amounts of cytochrome *c* and pyridine nucleotides [23], we compared mitochondria prepared from the hearts of normal, hyperthyroid and hypothyroid rats. A preliminary report has been made [18].

Materials and Methods

Three different groups of male rats (Spartan Company, Haslett, Michigan) were used, all fed a low-iodine, vitamin-enriched diet (Nutritional Biochemicals Corporation, Cleveland, Ohio) to validate comparisons. The diet contained, by our analyses, 7.9% total lipids on a dry weight basis, with 4.5% of 14:0; 17.1% of 16:0; 9.1% of 18:0; 39.3% of 18:1; 28.8% of 18:2; and 0.5% of 18:3. Group (a) were normal rats that received 0.0005% KI solution as drinking water. Group (b), thyrotoxic animals, also received KI and were injected intraperitoneally with 1 μg of L-triiodothyronine/g body weight each day for 3 days and killed on the fourth day. Group (c) were hypothyroid rats prepared by thyroidectomy together with the administration of ablative doses of ^{131}INa as in [22]; they received no KI and were maintained on the test diet for at least 3 weeks. All animals were fed *ad lib* to the time of killing.

Heart mitochondria were prepared in 0.25 M sucrose, using brief homogenization with a high-speed disintegrator (Tekmar Model 5DT), and centrifugal sedimentation of debris and nuclei at 600 **g** for 5 min; mitochondria were sedimented twice at 10 000 **g** for 10 min. Mitochondria prepared this way, as compared with those obtained by the Nagarse digestion of normal rat myocardium used in our previous study [36], showed higher initial respiratory control ratios, which were maintained longer on standing in iced suspension. The final mitochondrial pellet was suspended in 0.25 M sucrose at 20 mg protein/ml, after determining protein by a rapid biuret method [12] with bovine serum albumin for reference.

Respiration was measured with a Clark oxygen electrode and a Gilson Oxygraph, with appropriate corrections for O_2 solubility at different temperatures, in a 2.0-ml glass vessel constructed for a Peltier-effect thermoregulator with a thermal sensor in the reaction mixture to provide readout and feedback [20]. Temperatures were maintained within about 0.04°C during the 5 to 10 min required for measuring O_2-uptake rates, and temperature changes of 3 to 4°C became stable within a few minutes, which considerably shortened the time required for measuring respiration over the temperature range 7 to 40°C, for the construction of Arrhenius plots. The reaction mixture contained 0.25 M sucrose; 0.25 mM EDTA; 10 mM KCl; 5 mM potassium phosphate and 15 mM Tris buffer at pH 7.4; 20 mM glutamate; 2.0 mg mitochondrial protein; and 0.16 mM ADP added in microliter aliquots to determine State 3 respiration, respiratory control, and ADP/O ratios.

For assays of lipid composition, heart mitochondria suspensions were disintegrated by homogenization with 20 volumes of chloroform-methanol (2:1 v/v) containing 0.005% butylated hydroxytoluene. Total lipid extracts were resolved into neutral and phospholipids by passage down a silica gel column and elution with chloroform and methanol respectively. Phospholipids were further resolved by TLC [37], and the amounts of the major phospholipid classes* estimated from total phosphorus measurements [3]. Dry weights of lipid fractions were determined after removal of solvents under N_2 and *in vacuo*. Fatty acid compositions were measured, usually within a day after saponification and methanolysis at 70°C, by gas–liquid chromatography (glc) on a Varian model 3700 instrument, with FID at 250°C, injector at 220°C, using a 6′, 10% Silar-10C column temperature-programmed between 170 and 200°C at 3°C/min. Under these conditions, cholesterol was eluted just after the last fatty acid methyl ester (22:6) and cholesterol:FAME ratios (g/g) were calculated from peak areas and calibrations with pure compounds. Measurements chosen as optimal for cholesterol, using higher operating temperatures and a 2′, 3% OV-101 column [9], gave similar results (courtesy of Dr W. J. Ferrell).

Standards of 18:2, 18:3, 20:4 and 22:6 FAME (Nu-Chek-Prep, Inc., Elysian, Mn., USA), run daily, were recovered completely. Fatty acid assignments were made from comparisons with verified standards when available, or (e.g. 22:3)

* Abbreviations: Phosphatidylcholines, PC; phosphatidylethanolamines, PE; phosphatidylserines, PS; cardiolipins, CL; flame ionization detector, FID; fatty acid methyl esters, FAME; unsaturation index (Σ[percentage of unsaturated fatty acyl group × number of unsaturated bonds]), UI.

from interpolations of the logarithms of relative retention times of standards v. carbon numbers, according to Ackman [*1*]. Analyses of preparations from group (b) or (c) animals were paired with analyses on the control group (a), but the results are shown as group comparisons ± S.E. We have found that this procedure is important because control values change with season.

Results

Table 1 shows that intraperitoneal injection of 1 μg of L-triiodothyronine/g body weight/day for 3 days, and killing the animal on the fourth day, caused a significant loss of body weight; uninjected or saline-injected animals (not shown) did not lose weight. Hormone treatment increased the heart: body weight ratio by 37%, but decreased body weight by only 3.6%: we conclude that the absolute heart weight increased. Treatment did not significantly affect (+8%) the amount of heart mitochondria recovered/g of myocardium; recovery was quite reproducible, the coefficients of variation being 5.1% for controls, 10.6% for thyrotoxics.

Table 2 shows measurements of oxidative phosphorylation at 25°C in heart mitochondria from hormone-treated animals compared with those from controls. These thyrotoxic preparations respired at a normal rate in State 4 and at a slowed rate in State 3. Respiratory control was thereby depressed, although phosphorylative efficiency, as measured polarographically by ADP:O ratios, was unchanged. The respiratory depression but not the normal level of efficiency agreed with the findings of [*32*] after a 6-day hormone pretreatment.

The measurements in Table 2 were made within about 30 min after the final sedimented mitochondrial pellet was resuspended. We found that the time of standing in iced suspensions affected oxidative phosphorylation at 25° differently when mitochondria prepared from normal or thyrotoxic

TABLE 1. Body weight changes, heart:body weight ratios and recovery of heart mitochondria in control or thyrotoxic rats

	Controls (17 to 19)	Thyrotoxics (17 to 18)	*P*
Body weight change (g)	—	−8.4 ± 2.9	<0.025*
Heart:body weight (g/100 g)	0.404 ± 0.019	0.555 ± 0.026	<0.001
Mitochondria:heart weight (mg protein/g)	25.3 ± 1.3	27.4 ± 2.9	N.S.

* Paired comparisons of weights on day 1 of the 3-day treatment, and on day 4, when mitochondria were prepared. All values are means ± S.E., and the numbers of experiments are shown in parentheses. The controls weighed 306 ± 25 g, the thyrotoxics on day 1, 236 ± 17 g.

TABLE 2. Oxidative phosphorylation at 25°C in the heart mitochondria of normal (control) or thyrotoxic rats

	Controls (10)	Thyrotoxics (10)	*P*
State 4 respiration	44 ± 3.9	44 ± 3.9	N.S.
State 3 respiration	149 ± 11.0	101 ± 10.0	<0.001
Respiratory control	3.6 ± 0.48	2.4 ± 0.25	<0.05
ADP/O	1.6 ± 0.12	1.6 ± 0.13	N.S.

Respiration (ng atom O. min^{-1}. mg^{-1}; substrate, glutamate) is shown in State 3 (plus ADP and Pi) and State 4 (after phosphorylation of the added ADP). Respiratory control is the ratio State 3/State 4. The efficiency of phosphorylation is the ADP/O ratio, in mol. g $atom^{-1}$. All values are means ± S.E., the number of experiments is shown in parentheses, and *P* values are calculated by Student's *t* test.

rats were compared (Table 3). In thyrotoxic preparations the rate of respiration decreased linearly, and much more rapidly than in control preparations, as shown by the regression coefficients. State 3 respiration in the thyrotoxics decreased more rapidly with standing than State 4 respiration, which may be connected with the respiratory deficit shown in Table 2. On the other hand, both respiratory control and the efficiency of phosphorylation (ADP:O) decreased minimally if at all in both groups of mitochondria (note that these rates are min $\times$ 10^3 in Table 3). From preliminary data on the changes in State 3 respiration when the heart mitochondria of thyrotoxic rats stood in iced suspension for up to 100 min and respiration was assayed at temperatures between 5 and 37°C, we have concluded that the altered thermotropic respiratory responses may represent a change in membrane fluidity in the heart mitochondria of these thyrotoxic rats [*18*].

Among the lipid parameters shown to affect membrane fluidity and function [*22*] are lipid: protein ratios; phospholipid polar groups (phosphatidylcholines with weakly binding polar groups increase fluidity); fatty acyl unsaturation (directly related to fluidity); and cholesterol content (acts as a spacer molecule to alter fluidity). Analyses of lipid contents of heart mitochondria prepared from normal (control), thyrotoxic, or hypothyroid rats are presented in Tables 4 to 6.

Table 4 shows that the heart mitochondria of thyrotoxic rats contained excess neutral lipids and thereby excess total lipids, but had normal amounts of cholesterol and total phospholipids, and normal proportions of the resolved phospholipid classes. The mitochondria of hypothyroid rats did not show any abnormalities in these parameters.

Table 5 presents the fatty acyl contents, the unsaturation indices and the ratios of 20:4/18:2

TABLE 3. The time course of oxidative phosphorylation measured at 25°C, when heart mitochondria stood in iced suspensions

	Controls (3)	Thyrotoxics (3)	*P*
Δ State 3 respiration (min^{-1})	-0.19 ± 0.04	-0.73 ± 0.16	<0.05
Δ State 4 respiration (min^{-1})	-0.06 ± 0.01	-0.41 ± 0.11	<0.05
Δ Respiratory control (10^3 min^{-1})	-1.0 ± 0.6	-2.4 ± 1.3	N.S.
Δ ADP/O ratio (10^3 min^{-1})	-3.7 ± 2.7	-2.9 ± 4.7	N.S.

Mitochondria were prepared from each of three controls (n = 6 to 10 separate measurements made during 85 min following the resuspension of the mitochondrial pellet), and three thyrotoxic rats (n = 4 to 8 measurements during 110 min). The values shown are the means $\pm$ S.E. of the regression coefficients calculated by least squares from n points in each of the two sets of experiments. Respiration was measured in ng atoms $O \cdot min^{-1} \cdot mg^{-1}$; respiratory control is the ratio State 3:State 4 respiration; the ADP/O ratio was calculated from the polarographic tracings.

TABLE 4. Lipid composition of heart mitochondria prepared from normal (control), thyrotoxic or hypothyroid rats

	Controls (3 to 6)	Thyrotoxics (3 to 5)	Hypothyroids (4 to 6)
Total lipids (mg/mg protein)	0.25 ± 0.01	$0.35^* \pm 0.04$	0.31 ± 0.06
Neutral lipids (mg/mg protein)	0.07 ± 0.03	$0.16^* \pm 0.02$	0.11 ± 0.04
Cholesterol (g/g fatty acid methyl ester)	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02
Phospholipids (mg/mg)	0.18 ± 0.01	0.19 ± 0.03	0.19 ± 0.04
(%) Phosphatidylcholines	44.7 ± 1.5	45.4 ± 0.5	46.2 ± 9.1
Phosphatidylethanolamines	31.4 ± 4.0	28.8 ± 3.5	30.1 ± 6.2
Cardiolipins	15.5 ± 2.2	14.9 ± 0.3	14.5 ± 2.7
Phosphatidylserines	8.4 ± 6.0	11.0 ± 4.3	2.7 ± 0.3

The analytical procedures are described under Materials and Methods. All values are means $\pm$ S.E.; the number of experiments is shown in parentheses.

* $P < 0.05$.

TABLE 5. Fatty acid composition of the total lipids, neutral lipids and phospholipids extracted from the heart mitochondria of controls, thyrotoxic or hypothyroid rats

Fatty acyl	Total lipids			Neutral lipids			Phospholipids		
	Controls (5)	Thyrotoxics (5)	Hypothyroids (6)	Controls (7)	Thyrotoxics (6)	Hypothyroids (6)	Controls (7)	Thyrotoxics (6)	Hypothyroids (5)
16:0	9.8 ± 0.7	9.6 ± 0.6	10.2 ± 0.5	18.0 ± 1.1	18.9 ± 0.7	18.1 ± 1.2	9.1 ± 0.5	9.6 ± 0.3	10.6 ± 0.8
18:0	18.6 ± 0.5	18.9 ± 1.4	17.1 ± 0.7	6.6 ± 0.6	6.9 ± 0.5	9.3* ± 1.0	18.3 ± 0.4	18.8 ± 0.7	18.1 ± 0.7
18:1	17.1 ± 0.7	18.7 ± 0.9	16.0 ± 0.3	30.9 ± 0.9	35.5* ± 1.4	30.8 ± 1.7	14.4 ± 0.8	16.7* ± 0.4	13.8 ± 1.0
18:2	20.4 ± 0.6	21.4 ± 1.3	29.2*** ± 0.9	22.0 ± 0.9	22.8 ± 0.9	21.3 ± 1.2	20.4 ± 0.6	19.6 ± 0.7	28.1*** ± 0.8
20:4	21.4 ± 0.5	20.4 ± 0.9	17.3*** ± 0.6	3.7 ± 0.2	2.7* ± 0.2	3.3 ± 0.5	23.8 ± 0.5	22.1* ± 0.6	19.7*** ± 0.7
22:3	1.7 ± 0.1	1.7 ± 0.2	0.8*** ± 0.1				1.7 ± 0.3	2.0 ± 0.1	0.9* ± 0.1
22:4	4.1 ± 0.5	3.6 ± 0.2	1.3** ± 0.3				4.3 ± 0.4	3.7 ± 0.4	1.4* ± 0.4
22:5		0.5 ± 0.1	1.1 ± 0.2				1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.1
22:6	3.6 ± 0.6	3.3 ± 0.6	4.0 ± 0.4				5.1 ± 0.8	4.6 ± 0.8	4.0 ± 0.4
UI	194 ± 1.7	188 ± 3.4	185* ± 2.5	133 ± 6.3	129 ± 3.9	136 ± 8.6	209 ± 5.3	200 ± 3.5	190* ± 2.4
$\frac{20:4}{18:2}$	1.05 ± 0.02	0.97 ± 0.06	0.59*** ± 0.04	0.17 ± 0.01	0.12* ± 0.01	0.16 ± 0.03	1.17 ± 0.05	1.16 ± 0.03	0.71*** ± 0.04

Analytical procedures are described under Materials and Methods; n is shown in parentheses. Fatty acyl contents are shown as mean percentage of total fatty acids ± S.E.

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

TABLE 6. Fatty acid composition of the resolved phospholipid classes obtained from the heart mitochondria of control (C), thyrotoxic (T) or hypothyroid (H) rats

Fatty acyls	Phosphatidyl choline			Phosphatidyl ethanolamine		
	C	T	H	C	T	H
16:0	15.1 ± 1.3	16.8 ± 0.5	17.0 ± 0.8	9.1 ± 1.2	6.6 ± 0.4	9.1 ± 0.5
18:0	25.5 ± 2.8	25.1 ± 0.9	27.1 ± 1.9	29.0 ± 3.7	23.7 ± 1.3	20.3 ± 2.7
18:1	18.8 ± 1.2	19.9 ± 1.5	19.6 ± 1.6	17.2 ± 0.5	18.8 ± 0.7	16.0 ± 2.0
18:2	11.2 ± 0.4	11.2 ± 0.9	17.5** ± 1.1	5.7 ± 0.4	5.9 ± 0.5	9.8*** ± 1.5
20:4	23.0 ± 1.6	23.3 ± 0.6	12.4** ± 1.6	20.5 ± 1.2	21.8 ± 1.2	28.1* ± 2.6
22:3	1.5 ± 0.2		1.1 ± 0.2	2.7 ± 0.4	2.9 ± 0.6	2.2 ± 0.6
22:4	1.4 ± 0.3		0.9 ± 0.06	5.4 ± 0.4	8.0 ± 1.9	2.7 ± 0.9
22:5	0.8 ± 0.7		0.6 ± 0.03	1.7 ± 0.6	2.0 ± 0.4	2.2 ± 0.5
22:6	2.0 ± 0.6	1.3 ± 0.7	1.3 ± 0.03	7.9 ± 2.5	7.9 ± 1.3	5.9 ± 1.0
Unsaturation index	160 ± 12.4	152 ± 10.8	125 ± 9.1	199 ± 24.3	223 ± 9.0	223 ± 24.7
$\frac{20:4}{18:2}$	2.07 ± 0.21	2.3 ± 0.20	0.72** ± 0.10	3.90 ± 0.24	3.79 ± 0.41	3.09 ± 0.54

Fatty acyls	Cardiolipin			Phosphatidyl serine		
	C	T	H	C	T	H
16:0	1.2 ± 0.1	2.8 ± 0.9	3.1*** ± 0.3	4.1 ± 0.9	4.6 ± 0.5	4.7 ± 0.6
18:0	3.3 ± 0.3	3.7 ± 0.7	5.6 ± 0.6	35.5 ± 1.6	37.2 ± 2.9	35.9 ± 4.5
18:1	9.0 ± 0.7	11.6 ± 1.8	7.4 ± 0.6	18.0 ± 1.6	18.1 ± 1.8	15.6 ± 1.2
18:2	71.2 ± 5.0	64.0 ± 3.2	55.4 ± 6.0	4.5 ± 0.9	6.0 ± 1.9	5.8 ± 1.7
20:4	5.9 ± 2.3	3.9 ± 0.7	3.4 ± 0.5	19.0 ± 3.2	22.5 ± 3.5	15.3 ± 3.3
22:3	3.0 ± 1.8	3.6 ± 1.3	5.0 ± 1.5	2.3 ± 0.8	3.8 ± 1.8	6.6 ± 2.3
22:4	1.1 ± 1.0	1.0 ± 0.3	3.2 ± 1.5	3.5 ± 0.4	3.0 ± 0.4	2.9 ± 0.6
22:5				1.4 ± 0.8		
22:6	0.8 ± 0.6	1.4 ± 0.5	1.9 ± 0.8	2.1 ± 0.8		
Unsaturation index	195 ± 10.7	193 ± 13.2	213 ± 13.4	162 ± 14.4	154 ± 10.3	153 ± 17.3
$\frac{20:4}{18:2}$	0.09 ± 0.04	0.06 ± 0.01	0.07 ± 0.02	4.40 ± 0.27	4.85 ± 1.44	3.92 ± 2.19

For analytical procedures see under Materials and Methods; $n = 4$ for each group of mitochondria. Fatty acyl contents are shown as mean percentage of total fatty fatty acids ± S.E.

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

acyl groups in mitochondrial total lipids, neutral lipids and unresolved phospholipids. In preparations from the thyrotoxic rats, the total lipid fatty acyl distribution was similar to that in controls. The neutral lipids and phospholipids contained excess 18:1 and were slightly depleted in 20:4 acyl groups but not enough to depress the unsaturation indices. Hypothyroid heart mitochondria were strikingly abnormal in fatty acyl contents: the phospholipids had abnormally high proportions of 18:2 acyl groups and low amounts of 20:4, 22:3 and 22:4 acyl residues, which were also expressed in the depressed unsaturation index and 20:4/18:2 ratio. Our previous analyses of heart mitochondrial phospholipids in hypothyroid rats showed no significant decreases in 20:4 fatty acyl contents or in overall unsaturation [*36*]; this disparity is discussed below.

The fatty acyl contents of the resolved phospholipid classes (Table 6) were, as expected from the data in Table 5, at normal levels in the mitochondria of the thyrotoxic rats. In the hypothyroid rats, the PC fraction was seen to account for the overall deficiency of 20:4 fatty acyl moieties in the phospholipids (Table 5); the PE fraction actually contained more 20:4 than it did in control preparations. While both PC and PE in the hypothyroid mitochondria had excessive contents of 18:2 acyl groups, the PC fraction normally had a higher proportion of 18:2 than did PE, and PC was preponderant among the phospholipid classes (Table 4); PC fatty acyl abnormalities thereby accounted for most of the changes in the total phospholipids in hypothyroidism. Neither the CL nor the PS phospholipids contributed to the fatty acyl deviations.

Discussion

From the increased heart:body weight ratios in hormone-treated rats, which represented an absolute increase in heart weight (Table 1), it appears that cardiac hypertrophy was induced much earlier by our hormonal treatment schedule of 3 days of 1 μg of triiodothyronine/g than has been reported for other regimens. Gross and Pitt-Rivers [*13*] originally found an 87% increase in heart:body weight ratio 73 days after injecting triiodothyronine daily in a dose of about 0.2 μg/g; Gemmill [*10*] reported a 37% increase after feeding the hormone via the drinking water for 42 to 60 days; and Cohen [*8*] a 40% increase after 14 days of injecting mice with L-thyroxine, 1 μg/g day. On the other hand, we did not evoke mitochondrial proliferation with the 3 days of treatment, as judged from the reproducible preparative recovery of mitochondrial protein (Table 1). In contrast, other investigators have found chemical [*30*] and electron micrographic [*26*] evidence for increased numbers and size of mitochondria in the heart after 24 days of injecting L-thyroxine 0.18 μg/g every fourth day, and heart mitochondrial marker enzymes have increased only after 7 to 14 days of injection of about 1 μg of thyroxine/g every other day [*33*].

The relatively acute thyrotoxic state produced by our 3-day treatment decreased mitochondrial phosphorylating respiration at 25°C but did not depress phosphorylative efficiency (Tables 2 and 3). Our findings thus agree only in part with those of Piatnek-Leunissen and Leunissen [*32*] after a 6-day treatment, detailed in the Introduction, which serves to support their conclusion that the schedule of hormone treatment is crucial. However, State 4 respiration did not *increase* either in our studies (Tables 2 and 3) or in theirs, which is not characteristic of uncoupling of oxidative phosphorylation [*25*]. The respiratory deficit in State 3 suggests a loss of mitochondrial cofactors or electron transporters; early studies on liver mitochondria using very high thyroxine doses implicated NAD^+ and cytochrome *c*, and an increased membrane permeability [*21*], but no such data are as yet available on the heart mitochondria of our current thyrotoxic animals.

In view of the extensive evidence for changes in lipid contents affecting membrane function [*34*], the altered thermotropic respiratory responses of thyrotoxic heart mitochondria seem attributable to their increased neutral lipid contents (Table 4). In the absence of any change in cholesterol content, these were probably mainly triglycerides. Triglyceride content increased in the myocardium of rats injected with about 0.25 μg of thyroxine/g body weight per day for 10 days [*6*], and the hormone increased the rate of triglyceride synthesis, as observed in the hearts of rats injected with 0.5 μg of thyroxine/g/day for 5 days [*35*]. The neutral lipids of thyrotoxic mitochondria in our studies contained excess 18:1 acyl groups (Table 5). Since both thyrotoxic and control rats ate a diet high in 18:1 fatty acid content (39.3% of the total fatty acids), it seems likely that endogenously generated 18:1 was the source of the increased content in the thyrotoxic heart mitochondria. The heart depends on the liver for the necessary microsomal Δ9-desaturation to convert 18:0 to 18:1 [*4*], and we have found that our treatment schedule of injecting euthyroid rats with hormone increased hepatic Δ9-desaturase activity four-fold [*18*].

Heart mitochondria prepared from control rats contained 22:6 as 3.6% of total fatty acids (Table

5), and in previous studies, as 1.5% [*36*]. Other investigators have reported higher 22:6 fatty acid contents in heart muscle mitochondria or in heart muscle total lipids. We have reason to believe that the strain (i.e., the source) of rats, the diet fed, and the season, may all be connected with these differing compositions. Tissue 22:6, being of the ω3 fatty acid family [*5*], is not biosynthesized by rats from endogenous sources. Dietary ω3 fatty acids incorporate readily into heart lipids, especially when the essential ω6 fatty acids are supplied at low levels [*14*]. The synthetic diets for our rats contained 18:3 as 0.5% of total fatty acids and as the only ω3 acid, and 28.8% of 18:2 as the only ω6 fatty acid. About 5% of 22:6 was found in heart mitochondrial fatty acids from rats on a diet containing 18:3 as 2% and 18:2 as 55.6% of fatty acids [*38*], and 10% 22:6 occurred in heart total lipids in rats fed a diet with 18:3 as 10% and 18:2 as 10% of fatty acids [*31*]. Even higher proportions, 22:6 = 12.1% of fatty acids, were seen in heart lipids of rats fed a diet containing 10% cod liver oil; this oil contains 22:6 as 10% and 20:5ω3 as 10%, but 18:2 as only 2%, of its fatty acids [*14*]. Many investigators have not specified the diet used or its fatty acid content. For example, heart mitochondrial phospholipid fatty acids were reported to contain 9.7% of 22:6, with 21.5% 22:6 in the PE fatty acids [*11*]. We found 22:6 as 7.9% of the PE fatty acids (Table 6), which incidentally confirms that our analytical procedures do recover 22:6. Percentage compositions as high as 18.5% and 12.6% of 22:6 in heart mitochondrial inner membrane lipids have been reported [*15, 29*]. In view of the susceptibility of 22:6 content, and perhaps that of other fatty acids, to exogenous variables, we have relied on paired experiments done on litter-mates kept on a diet of analyzed fatty acid content. The same reasoning seems valid for comparisons of mitochondrial function: the respiratory control ratio of 3.6, measured at 25°C with glutamate as substrate (Table 2) compares with our previously reported ratio of 4.3 measured at 32°C [*36*], although values greater than 5 have been reported for heart mitochondria prepared from other rats on other diets, measured under other conditions.

Buccino *et al.* [*7*] observed an abnormally rapid rate of tension-development and an abbreviated tension-maintenance time in the papillary muscles of thyroid-treated cats at 37°C. They also found a hormone-induced alteration of some thermotropic contraction property, in that a decrease of temperature to 21°C slowed the rate of tension-development and prolonged tension-maintenance more in thyrotoxic muscles than in control muscles. The nature of that temperature-sensitive process is not as yet clear. From our studies, an altered mitochondrial energy supply might be involved. However, Alpert and Mulieri [*2*], working with hypertrophied heart muscles of rabbits pretreated with hormone for 2 weeks, concluded that the thyroid-induced alteration of the myosin primary structure [*40*] affected the rate-limiting step of the actomyosin-ATPase cycle to account for the contractile changes. No thermotropic properties of "thyrotoxic myosin" have been reported. Observations on hepatic endoplasmic reticulum lipids [*22*] suggest that hormone-induced changes in the myocardial sarcoplasmic reticulum, which also controls shortening velocity and maximal tension by removing Ca^{2+} ions from the Ca^{2+}-troponin complex [*24*], might be involved. Several reports support a contribution of this membrane pump: its membrane-dependency altered thermotropically with lipid composition [*16*]; its activity at 30°C [*41*] and its capacity at 37°C [*16*] were increased in microsomes from thyrotoxic hearts; and the increase of myocardial diastolic tonus in rabbit heart papillary muscles at low temperatures has been attributed to the temperature-senstivity of the Ca-sequestration system [*39*]. Hormonal effects on the temperature-dependency and composition of this membrane system are currently under study.

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